[0290] At 70% confluence, osteogenic media (basal media plus 10 nmol/L dexamethasone plus 100 nmol/L ascorbate-2-phosphate) was substituted and after a further 24 hours, cells were gently trypsinised, counted and resuspended in osteogenic media in preparation for seeding onto material samples.

[0291] Tissue culture reagents were obtained from Gibco/BRL (Paisley, Scotland). Reagents were of analytical grade from Sigma Chemical (Poole, UK) unless otherwise stated.

[0292] 3 mm cubes of fully hydrated autoclaved material were soaked in basal media for 24 hours and transferred to 24-well tissue culture plates.

[0293] 48 hours prior to implantation, 10 μl of a cell suspension [1×104 cells] of adult human bone marrow stromal cells was pipetted onto each cube and incubated at 37° C. for 30 minutes before 1 ml of osteogenic media was added to each well.

[0294] Unseeded material samples were used as controls.

[0295] After specified intervals, material was fixed in buffered formaldehyde solution and embedded in methacrylate resin. 10 μm sections were cut with a tungsten knife.

[0296] Fluorescent staining with cell tracker green and ethidium homodimer-1, as well as histological staining with haematoxylin and eosin, indicated the presence of viable HBMSCs (human bone marrow stromal cells) within the pores and on the surface of the fibroin-apatite material. Inward growth of the cells was visible by day three and complete colonisation of the porous monolith observed after seven days.

[0297] The HBMSCs remained viable over three weeks in culture, with maintenance of the osteoblast phenotype within the material, as evidenced by type I collagen and alkaline phosphatase immunocytochemistry.

Example 7

Protocol for Testing Fibroin-Apatite Materials

In Vivo Testing

[0298] 3 mm cubes of fully hydrated autoclaved material were seeded with human bone marrow stromal cells. They were implanted without prior incubation subcutaneously into eight immunocompromised MFI nu/nu mice under anaesthesia.

[0299] Seeded samples were placed in the left flank and unseeded controls in the right flank of each animal Mice were left for 4, 8 and 12 weeks before sacrifice.

[0300] Haematoxylin and eosin stained glycol methacrylate resin sections of cell-seeded material taken from the mice were examined (FIGS. **12-15**). After eight weeks, the sections showed the presence of newly formed bone secreted by osteoblasts on the surface of the porous material. Palisades of osteoblasts were observed on the osteoid surface and connective tissue scale. Evidence of remodeling of the newly formed bone by multinucleate osteoclasts was observed on the surface of the osteoid matrix. No evidence of adverse cell or tissue reactions was observed in seeded and unseeded controls.

[0301] These observations together with those of in vitro testing described above, demonstrate the excellent biocompatibility of the di-isocyanate cross-linked material. The observations made on in vivo testing further strongly suggests that the material is highly osteogenic, that the material is slowly resorbed and that the bone formed de novo in the material undergoes remodeling.

Observations

[0302] The porous, resorbable, biocompatible, pyrogen-free, implantable material described above is highly advantageous, because it combines the properties of compressive strength, compressive elastic modulus and compressive toughness close to that of previously defined target valves with an appropriate resorption rate and excellent tissue regenerative properties. These properties make the material suitable for all immediate and non-immediate load-bearing applications, non-load-bearing applications and as a substitute for allograft and autograft bone.

[0303] The similarity of the mechanical properties of the implantable material to those of natural bone make the material capable of immediately bearing the stresses to which bones are subjected in normal movement, thereby avoiding the need for prolonged periods of bed rest and minimizing the use of internal or external supports. The implantable material can therefore, be used in load-bearing implant locations to replace all or a part of a bone, or to lie between a bone and a metallic or ceramic or plastic prosthesis.

[0304] The exceptional toughness of the implantable material makes it particularly suited to impaction grafting, because the pores are protected from collapse during impaction allowing for rapid ingress of cells and blood vessels. Therefore, the implantable material can also be used to fill voids in bones.

[0305] The high and open porosity and large mean pore size of the implantable material enables mesenchymal stem cells, osteoblasts, osteoclasts and developing capillaries to migrate into the material initiating the materials conversion to natural bone. This together with the excellent biocompatibility and adhesiveness for cells of the implantable material allows cells to adhere, grow and differentiate within the pores of the material enabling the rapid de novo production of bone.

[0306] The slow resorbability of the implantable material enables it to be gradually and completely replaced by functional endogenous bone.

1. A method for the preparation of an implantable material for the repair, augmentation or replacement of bone from a fibroin solution, the method comprising the steps of:

preparing a gel from the fibroin solution; and

preparing a material by subjecting the gel to one or more steps of freezing and thawing the gel,

wherein the step of preparing the gel from the fibroin solution is performed in the presence of phosphate ions.

2. The method according to claim **1**, wherein the method comprises the further step of subsequently treating the material with a cross-linking agent.

3. A method for the preparation of an implantable material for the repair, augmentation or replacement of bone from a fibroin solution, the method comprising the steps of:

preparing a gel from the fibroin solution;

preparing a material by subjecting the gel to one or more steps of freezing and thawing the gel,

wherein the method comprises the further step of subsequently treating the material with an isocyanate.

4. The method according to claim **3**, wherein the gel is treated with phosphate ions, or the step of preparing the gel from the fibroin solution is performed in the presence of phosphate ions.

5. (canceled)